Arnold Schwarzenegger
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DETERMINING HOW FISH DETECT FISH SCREENS AND TESTING POTENTIAL FISH SCREEN ENHANCEMENTS

PIER FINAL PROJECT REPORT

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Preface

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

The PIER Program, managed by the California Energy Commission (Energy Commission), conducts public interest research, development, and demonstration (RD&D) projects to benefit California.

The PIER Program strives to conduct the most promising public interest energy research by partnering with RD&D entities, including individuals, businesses, utilities, and public or private research institutions.

PIER funding efforts are focused on the following RD&D program areas:

- Buildings End-Use Energy Efficiency
- Energy Innovations Small Grants
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- Environmentally Preferred Advanced Generation
- Industrial/Agricultural/Water End-Use Energy Efficiency
- Renewable Energy Technologies
- Transportation

Determining How Fish Detect Fish Screens and Testing Potential Fish Screen Enhancements is the final report for the How Fish Sense the Presence of Fish Screens project (contract number 500-02-004, WA No. MR-035) conducted by the University of California, Davis. The information from this project contributes to PIER's Energy-Related Environmental Research Program.

For more information about the PIER Program, please visit the Energy Commission's website at www.energy.ca.gov/pier or contact the Energy Commission at 916-654-5164.

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Abstract

The importance of vision and the lateral line system in fish-screen avoidance was investigated in steelhead trout (*Oncorhynchus mykiss*). Fish were viewed swimming in front of a screen while in a large laboratory flume. Experiments were conducted during the day (lighted) and night (dark, to limit vision), and streptomycin sulfate treatments were used to block mechanoreception. The average number of times a fish contacted the screen was analyzed as a measure of performance. Industrial vibrators were used to induce screen vibrations as a possible behavioral deterrent. Fish showed a trend of contacting the screens less frequently during the day and more frequently when their lateral line system was blocked, although differences among the treatments were statistically indistinguishable ($P > 0.05$, three-way analysis of variance). At the frequency tested, the vibrations on the screen had no effect on the fish's avoidance performance. Recommendations for future experiments are discussed.

Keywords: Fish screen, steelhead trout, *Oncorhynchus mykiss*, mechanoreception, lateral line system, neuromast, fish entrainment, fish impingement, water diversion, behavioral deterrence

Executive Summary

Introduction

The water diversions needed for hydroelectric plants, agriculture, and residential water supply can be lethal to fish—including endangered and threatened species—that become entrained in their influent current. Wire-mesh screens are commonly situated in front of water diversions to prevent fish from being displaced from their habitat, but the protective screens themselves can be fatal if fish contact them severely or repeatedly.

Fish screens can be modified to provide better "warning" to fish, making it easier for fish to detect the screen in time to avoid contact. Designing deterrent fish screens requires a better understanding of how different species of fish sense the presence of the screens.

Fish commonly use vision to maintain swimming position and recognize obstructions in the water column. The other sensory input likely to affect swimming performance is mechanoreception, which allows a fish to detect movements and vibrations in the water surrounding its body by using its lateral line system. Though all fish use the same sensory systems to monitor their surroundings and maintain a regular swimming position, the sensitivity and importance of the systems can vary among species or under different conditions.

Purpose

The purpose of this project was two-fold: (1) to determine the relative importance of vision and mechanoreception (lateral line system) in screen avoidance for steelhead trout, and (2) to determine if fish screen designs that produce increased stimuli are more effective at deterring contact.

Objectives

This project worked with juvenile, captive-reared steelhead trout, a threatened native species that encounters many water diversions along its California migration routes. Swim tank experiments, stereo-fluorescent microscopy, and scanning electron microscopy were used to accomplish the following specific objectives:

1. **Effectively image the lateral line system.** A fluorescent DASPEI stain technique was successfully used to view the lateral line system of juvenile steelhead trout under a stereo-fluorescent microscope. Once the location of lateral line cells (neuromasts) was known, unstained fish heads were examined under a scanning electron microscope to view the individual neuromast cells in detail.
2. **Determine relative importance of vision and mechanoreception to screen avoidance.** The contribution of vision was tested via swim trials in the dark (night) and light (day). The contribution of mechanoreception was tested by dosing some fish with 0.02 g/L streptomycin sulfate, an antibiotic that has been shown to temporarily inactivate the neuromast cells of the lateral line system, thereby impeding the fish's sense of mechanoreception (Blaxter and Fuiman 1989; Montgomery et al. 2003).

The screen avoidance experiments were conducted in a custom-built, flume-style swim chamber where fish were viewed and video-recorded as they swam repeatedly past wedge-wire fish screens. For each set of test conditions, eight individual steelhead were tested separately for 15 minutes apiece.

3. **Determine the effectiveness of vibrating screens.** To test the hypothesis that increasing the intensity of perceivable stimuli emanating from a fish screen can allow fish to perceive its threat at a greater distance and thereby avoid contact, this project tested the effect of vibrating the screens at 45 hertz in swim tank trials as described above. The project had originally planned to test strobe lights as well, but ran out of time.
4. **Assess streptomycin effects on neuromast (lateral line) cells.** In addition to investigating the behavioral effects of streptomycin via swim trials (Objective 2), the project imaged streptomycin-treated neuromasts with scanning electron microscopy. Juvenile steelhead—different from the swim-trial steelhead—were treated with varying doses of streptomycin sulfate: 0.000 g/L (control), 0.010 g/L, 0.020 g/L, 0.050 g/L, and 0.100 g/L. The fish were then fixed, dehydrated, gold-sputtered, and examined under SEM to view the effects of the antibiotic on neuromast hair structures.

Outcomes

Fish contacted the screen during all treatments. A three-way analysis of variance (ANOVA) analyzing the effects of light level, streptomycin exposure, and screen vibrations found no significant differences between any of the treatments ($P > 0.05$). There was much individual variation in the swimming performance trials of all treatments, most noticeably in the control group. Some steelhead chose not to swim and contacted the screen frequently or impinged on it, while others swam at the front of the chamber and never approached the screen. The individual variation was greater than the variation caused by the treatments, adding to the lack of significance in the findings.

Nonetheless, swim performance was generally better in the light than in the dark, and without streptomycin, indicating that steelhead use both vision and mechanoreception to avoid screen contact. Vision appears to predominate in this species.

Yet the effect of streptomycin on the steelhead lateral line system was not clear. The SEM images showed no indication that the fishes' neuromast (lateral line) cells were damaged, even at 10 times the dose that has been shown to affect trout entrainment in past studies.

Conclusions and Recommendations

Although the differences between treatments were not statistically significant, they indicate that juvenile steelhead trout may use a combination of vision and their lateral line system to detect and avoid fish screens during the day, and the lateral line is likely to be guiding their responses at night.

However, the contribution of mechanoreception is not clear, as the SEM studies showed no damage to the neuromasts. Possibly, the neuromast hair cells regenerated within the 24-hour exposure period, or a buildup of nitrogenous fish waste in the bucket inactivated the antibiotic. Because the streptomycin-treated fish appeared to contact the screen more severely and to swim in a more startled manner at night, compared with the non-treated fish, it is possible that the streptomycin blocked some lateral line sensory input, but by some other means than hair cell cleavage. An electrophysiological study monitoring the neural responses in the lateral line system of fish treated with streptomycin (compared to a control group) would be needed to determine the antibiotic's effect.

Screen vibrations at 45 hertz had little effect on swimming performance. Other frequencies may invoke a greater response from the fish, as may randomized bursts of vibrations. Other species may also respond to the vibrating stimulus to a greater extent. This study determined that trout have very few neuromasts in their lateral line system compared to cyprinids (minnows and carps) or centrarchids (sunfish and bass), and may not be as attuned to vibrational cues in their environment. Other stimuli, such as strobe lights reflecting off the screen, may prove to be a more effective deterrent for a predominantly visual fish such as steelhead. It is also possible that steelhead born and reared in natural settings with predators would respond differently than captivity-reared fish to unusual vibrations in the water or the presence of a screen.

Recommendations

Various behavioral fish screen deterrents remain to be tested. The vibrations emitted from the screen can be tested at different frequencies and amplitudes. Non-constant, randomized bursts of vibrations may provide the perception of a greater threat or may effectively prolong fish habituation to the stimulus. Strobe lights may also provide a strong visual deterrent, particularly at night. Some of the larger water-extraction facilities in California use louver arrays instead of fish screens; vibrating louvers may be more effective than vibrating screens.

Because different species of fish can have vastly different abilities to perceive vibrations at different wavelengths and amplitudes, tests should be conducted on other fish species of interest, such as Sacramento splittail or delta smelt—as well as marine species, which may become entrained in the influent of coastal power plants that use once-through cooling.

The authors will continue this research on different species of freshwater and marine fishes and test the effectiveness of vibrating or illuminated screens and louvers as behavioral deterrents.

Benefits to California

California's demand for water and electricity is expected to increase in the near future, increasing the need for water diversions and the concomitant threat of entrainment-related losses for aquatic animals. Fish screens with improved deterrent capabilities will help minimize the impact of water diversions on California's aquatic populations.

The equipment and ideas derived from this project will guide further research on an effective, close-proximity fish-screen deterrent. The specific knowledge gained about steelhead trout is particularly valuable, as steelhead represent an important, threatened gamefish, which can encounter many fish screens at industrial and agricultural water diversions during their migration to the ocean.

1.0 Introduction

1.1. Background and Overview

Water diversions are necessary to help supply California's electrical, agricultural, and residential demands. However, these water diversions can be lethal to residential and migratory species, including many species of endangered and threatened fish, that can become entrained in their influent current. Fish screens are situated in front of many water diversions to prevent fish from being displaced from their habitat, but the screens themselves can be fatal if fish contact them severely or repeatedly. To improve their effectiveness, fish screens may be modified to make their presence widely apparent to different fish species, providing them the greatest chance for avoiding contact. California water demands are expected to increase in the near future, increasing the threat of entrainment-related losses for many aquatic animals. An increase in screen efficiency may help to minimize the current and future impacts of water diversions on California fish populations.

To pass a fish screen without contact, fish must be able to both detect the screen's presence and avoid it. All fish use the same sensory systems to monitor their surroundings and maintain a regular swimming position, but the sensitivity and importance of the systems can vary among species or under different conditions. Vision is commonly used to maintain swimming position and recognize obstructions in the water column (Herman et al. 1996; Boyd et al. 2001). Fish have the greatest diversity in visual capabilities and visual anatomy among vertebrates, and the visual abilities of some fish change throughout their life (Kusmic and Gualtieri 2000).

The other sensory input that is likely to affect swimming performance is mechanoreception. This sense allows a fish to detect movements and vibrations in the water surrounding its body, by using its lateral line system. The lateral line functions by detecting the presence, velocity changes, and strength of water flows, which can be as important to swimming orientation as gravity and light (Montgomery et al. 2000). It is used by some fish species to detect structures (Montgomery et al. 2001), avoid predation (Canfield and Rose 1996), maintain school formations (Pitcher and Perrish 1993), and orient to water flows (Bleckmann 1993). Differences in sensory capabilities among species are theoretically the result of evolution to different habitats (Kusmic and Gualtieri 2000; Montgomery et al. 1993) and may govern the species' ability to detect and avoid fish screens.

The lateral line system is composed of both superficial and canal neuromasts, which are specialized cells in the fish's skin used to detect vibrations. Superficial neuromasts are located on the surface of the fish's skin and are covered by a cupulae, or gelatinous sacs filled with cellular fluid, which project outward from the fish's body. The cupulae are bound to the water molecules surrounding them, causing them to tilt and detect vibrations and water movements near the fish's body. This tilting increases or decreases an ongoing neuronal signal (based on the direction the cupulae are bent) to the fish's brain (Engelmann et al. 2002). By integrating numerous neuromasts, which are sensitive to displacements in different directions, a fish can determine the speed, direction, and turbulence of the water surrounding it, allowing the fish to perform rheotaxis (a fish's ability to orient its body toward a water current) and other important swimming behaviors (Voigt et al. 2000; Carton and Montgomery 2002).

Canal neuromasts are located slightly beneath the fish's skin in canals that are open to the water through a series of small pores and commonly develop along the face and the sides of a fish's body. Canal neuromasts have evolved to determine acceleration in water currents and the locations of other moving objects in the fish's vicinity. By being located in canals, the canal neuromasts are shielded from stimuli generated by constant water velocities, allowing them to detect high-frequency wavelengths generated by accelerations or decelerations in flow. These stimuli trigger temporary increases in the ongoing neuronal responses. Therefore, canal neuromasts can detect wavelengths from external sources (e.g., vibrating spheres—and, possibly, vibrating screens) equally well in still and flowing water. Superficial neuromasts show a greater response to vibrating spheres in still water, but almost no response in fast currents (Engelmann et al. 2002; Krother et al. 2002).

Researchers have used antibiotics to pharmacologically block the function of fishes' lateral line systems and determine their importance for specific behaviors. The entire lateral line system can be blocked by the application of streptomycin (an antibiotic) to the fish's water supply (Blaxter and Fuiman 1989). This treatment may cleave off the hair cells from the outside of the fish's neuromasts, making them non-functional, as has been confirmed by scanning electron microscopy (SEM) for the antibiotic gentamicin sulfate (Song et al. 1995). Fish subjected to this treatment lose the ability to orient to low-velocity water flows or to locate external vibrating stimuli (Montgomery et al. 1997, but see Jassen 2000). Behavioral studies of this type have many advantages. First, they commonly yield responses at lower thresholds than found in studies using electrophysiological readings of afferent neurons (Montgomery et al. 2000). Second, the studies only affect fish temporarily, because their hair cells will grow back within a few days after treatment. And third, they are performed on free-swimming fish, allowing otherwise natural behaviors during specific activities, including swimming past a fish screen.

The contributions of vision and mechanoreception to screen recognition can be determined through straightforward screen avoidance experiments Fish can be tested in both light and dark conditions to determine the importance of vision to screen avoidance. Previous studies have determined that many fish species contact screens less frequently and severely under lighted conditions (Swanson et al. 2004), suggesting that visual recognition of screens is a limiting factor in screen detection. To test the importance of mechanoreception, fishes' lateral line systems can be temporally blocked by placing them in water containing antibiotics, allowing otherwise natural behaviors to be observed. Treated fish can be compared to control (untreated fish) to determine the role of mechanoreception in screen avoidance.

It may be possible to enhance a screen's detectability by increasing specific stimuli it generates. Implementing artificial lighting or vibration generators along the screen might enhance fishes' abilities to recognize and safely pass the screens. Other experiments attempting to use infrasound and flashing strobe lights, to behaviorally deter fish from hazardous areas in natural environments, have found mixed results. At night, juvenile salmon have been shown to swim away from strobe lights with tests conducted in lakes (Maiolie et al. 2001), net pens (Ploskey and Johnson 2001), and laboratory settings (Mueller et al. 2001). Other studies determined that salmon avoid strobe lights used during the day, and steelhead are unaffected by them (Taft et al. 2001). Speakers placed in the environment broadcasting different wavelengths of infrasound have led to very strong avoidance by juvenile salmon in some experiments (Sand et al. 2001), and no significant effects in others (Gotez et al. 2001).

Whereas past studies used intense sensory stimuli generated by small, nonthreatening objects to frighten fish away from areas, this project increased the stimuli generated by a large object in the environment that fish should perceive as truly threatening (a large fish screen). A fish will naturally avoid contact with a screen when possible (Swanson et al. 2004). Fish should respond to experimentally generated infrasound and vibrations, because these will be generated as ("near-field") particle accelerations, rather than distant ("far-field") vibrations, that fish can recognize as a proximal, directional threat. In past studies, this type of infrasound has been the most effective in deterring fish (Sand et al. 2001). The goals of this experiment were to enhance fish screen detectability and its potentially perceived threat level, and to minimize fish contact with a screen.

Enhancing the screen's detectability may increase other natural dangers that fish, particularly small fish, face in the habitat. Visual piscivores (fish-eating animals) may aggregate in areas with increased illumination, or become more effective at capturing prey. Because different fish species are known to detect different wavelengths of light and ranges of vibration frequencies, it may be possible to isolate stimuli that would increase the detectability of the fish screen to vulnerable species and be of little use to predatory fish. For example, two of California's native intertidal fishes (staghorn sculpin, *Leptocottus armatus*, and plainfin midshipman, *Porichthys notatus*) have very different abilities to see in long-wavelength (red) light (Mussen and Cech, in review a, b).

1.2. Project Objectives

The objectives of this project were to determine the sensory stimuli that steelhead trout use to recognize the presence and threat of a fish screen, and to suggest new screen modifications that will improve fish passage safety by fish screens. The project tested the hypothesis that steelhead use a combination of visual and mechanosensory (via the lateral line system) information to recognize and avoid fish screens, and identified the relative importance of each for the avoidance behavior. Furthermore, the project tested the hypothesis that increasing the intensity of perceivable mechanosensory stimuli generated by a fish screen can allow fish to perceive its threat at a greater distance, presumably decreasing contacts with the screen. The effect of visual enhancement (from strobe lights) was omitted from the experiment, due to insufficient time. Extra time was needed at the start of the project period to assemble and calibrate the experimental swimming chamber.

2.0 Methods

Research was conducted at the Center for Aquaculture and Aquatic Biology (CABA) on the University of California at Davis campus. Juvenile steelhead trout (*Oncorhynchus mykiss*) were collected from the Nimbus Fish Hatchery (located in Rancho Cordova, California) and transported to UC Davis where they were fed and maintained daily in flow-through, aerated, and temperature-controlled tanks.

2.1. Locating and Imaging Lateral Line Neuromasts

The location of lateral line neuromasts was determined by viewing anesthetized steelhead trout juveniles under a stereo-fluorescent microscope equipped with a special microscope filter (FITC, a filter designed for viewing fluorescein isothiocyanate), which only allows green wavelengths of light to be viewed. The fish were treated with a vital stain, 1 mmol DASPEI/L, for 30 minutes, causing their neuromasts to fluoresce and emit green light. DASPEI—i.e., 2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide—comes as a powder that can be dissolved into water; it has an excitation of 440 nm and an emission of 530 nm. The lateral line neuromasts of Chinook salmon juveniles were also successfully imaged in the same manner, although the project focused on steelhead trout rather than salmon.

Once the location of the superficial and canal neuromasts was identified, unstained trout heads were sampled to view the hair cells of neuromasts under a scanning electron microscope (SEM). Although many of the preliminary SEM sample preparation techniques produced poor-quality samples, eventually a process was developed that allowed clear views of the fish's skin and half of the fish's neuromasts. The successful fixation process started with anesthetizing the fish in a chilled solution of buffered tricaine methane sulfonate, or MS-222 (0.05 g MS-222, 0.10 g sodium bicarbonate, and 3.00 g sodium chloride/L) and bisecting the fish's head behind the operculum using a razor blade. (Opercula are the hard structures covering fish gills that both protect the fragile gills and help fish ventilate.) The head was then rinsed, by agitating in a bucket of water, and placed into a chilled vial of Karnovsky's reagent, pH-8, to preserve the tissue. Karnovsky's reagent is a phosphoric saltwater solution, which contains 2% gluteraldahyde and 1.5% paraformaldehyde. After more than 24 hours of fixation, the head was dehydrated in a graded series of ethyl alcohol and Hexamethyldisilizane (HMDS), a highly evaporative liquid. Specifically, samples were chilled and submerged for one hour in 15%, 30%, 50%, 75%, 90%, 95%, and 100% ethyl alcohol, followed by half 100% ethanol and half HMDS for one hour and finally HMDS for 36 hours. The HMDS was poured off, and samples were air dried. The dry samples were affixed to aluminum SEM specimen stubs with Duco Cement mixed with carbon dust, and gold sputtered. The specimens were then examined with a scanning electron microscope, so that images of hair cells could be viewed and recorded.

2.2. Determining Streptomycin Dose

To determine the required streptomycin concentration needed to eliminate lateral line function in juvenile steelhead, a pilot study was conducted in which fish were held in individual buckets holding 10 liters of water and one of four concentrations (plus one control) of streptomycin for 24 hours (Blaxter and Fuiman 1989), then sacrificed and fixed for SEM viewing as described above. Because a streptomycin dose of 0.011g/L has been shown to disrupt trout lateral line function in the dark (Montgomery et al. 2003), the doses tested were 0.000 (control), 0.010, 0.020, 0.050, and 0.100 g streptomycin/L.

2.3. Swimming Chamber Construction

A custom fiberglass swimming chamber (flume) was designed and constructed with the assistance of D&T Fiberglass of Sacramento, California. The flume is 25 feet long, 10.5 feet wide, and 3 feet deep, with a 3-foot-wide, 3-foot-deep channel. A 7.5-horsepower, 320-volt GOULDS pump was installed with an Automation Direct variable frequency drive (VFD), to vary the pump's speed. Four stainless steel, wedge-wire fish screens (3 feet by 3 feet) were installed in one of the flume's straight channels, approximating an hourglass shape with a narrow passage for fish to pass through while circulating the flume (Figure 1).

Initial trials found that the water velocity was much greater along the outer walls of the flume and aluminum turning vanes needed to be added at the curved end of the flume, upstream of the screens, to distribute the flow evenly over the wedge-wire screens. During pilot-level swimming experiments after the addition of the turning vanes, the fish began holding station between near the aluminum vanes at all water velocities, thereby ceasing to interact with the screens. Therefore, the screen design was revised so that two wedge-wire screens were used, with one end of the screens meeting in the middle of the flume and the other ends coming close to the opposite walls of the flume, creating a triangle shape. An additional screen made of one-quarter-inch stainless steel mesh held to a steel frame was added, upstream of the wedge-wire screen, allowing fish to be contained in front of the view area. This configuration exposed the fish to contact with the one of the wedge-wire screens if it drifted backwards in the flume during swimming trials, but it allowed the fish an open area ahead of the these screens where it could swim to avoid contact.

Figure 1. Original screen orientation

Figure 2. Final screen orientation

Individual Houston BV-150 industrial vibrators were bolted to each of the wedge-wire screens to test the effect of added vibrations. These vibrators are pneumatic piston-driven vibrators that can be used underwater. At 20 psi air pressure, they vibrated at 45 hertz. During the swimming trials the vibrators were located in the middle of the screens, just above the water level. The water depth was maintained at 30 cm (11.8 inches) and the temperature was maintained at 13°C (55°F).

A daytime and an infrared camera were suspended from the ceiling above the chamber in the area where the fish would be swimming. This allowed the behavior to be filed on a VCR for later detailed analysis. Two infrared LED floodlights were installed next to the cameras to illuminate the chamber during swimming trials in the dark. A clear plexiglas view plate was designed and constructed to float on the surface of the water in the area 1 inch upstream of the wedge-wire screens, so fish behavior could be viewed without distortion.

2.4. Swimming Trials Procedures

The time for experiments was brief, due to a lengthy construction time. Therefore, one experimental set, with eight replicates, was conducted to test how added vibrations, streptomycin treatments, and time of day affected the contact rate of steelhead against fish screens.

Individual steelhead were placed in 5-gallon buckets filled with 15 liters of water, and held for 24 hours in a chilled water bath to maintain the 13°C temperature. Because the results of SEM streptomycin pilot study (Section 2.2) showed no visible impact of the antibiotic on neuromast hair cells, pilot study results could not be used to determine the appropriate streptomycin dose for the swim trials. Instead, the dose was selected on the basis of previous research. Buckets either had 0.02 g/L streptomycin sulfate added to them, which has previously been used to block the entire lateral line system of trout (Montgomery et al. 2003), or no chemical (control).

Individual fish were netted from the bucket and rapidly transported into the swimming chamber. Each fish was given an hour of acclimation to the chamber, then the pump was turned on starting a current of 60 cm/s (± 6 cm/s) in the chamber. The vibrator was started at the same time as the pump if it was used during the trial. Pilot studies determined steelhead could easily maintain their position and swim forward into the current at this water speed. The fish's swimming was viewed and recorded for 15 minutes. After the trial the fish was netted from the chamber, euthanized in ms-222, and its length and mass measured. The same procedure was used during day and night experiments for all of the treatments (day/night, streptomycin/no streptomycin, and vibrations/no vibrations). Eight fish were tested for each of the eight treatments, resulting in a total of 64 fish tested.

3.0 Results

3.1. Results of the Microscope Studies

The DASPEI vital stain clearly fluoresced all of the fish's lateral line neuromasts. The testing revealed that steelhead trout have very few superficial neuromasts, located in two rows along the fish's head and around the fish's nares. No superficial neuromasts were found along the trunk or fins of the fish. Superficial neuromasts are visible as the small green dots in Figure 3. The canal neuromasts beneath the fish's skin were also visible, and were located on the head and along the lateral line of the trunk. The canal neuromasts are visible as the large green dots in Figure 3. These findings are supported by those of Montgomery et al. (2003).

Once the locations of superficial neuromasts were known, they were easy to locate on the prepared SEM samples. The final fixative used on the SEM samples often provided very clear views of the fish's skin, but many of the neuromasts were covered in a substance that blocked the view of the hair cells. The substance, which was removed during some fixations, may have been part of the cupula, the gelatinous sac that covers the neuromast while the fish is alive.

Interestingly, on the uncovered neuromasts, the SEM study found that streptomycin sulfate had no distinguishable effect on steelhead hair cells at any of the tested concentrations. Shown below are neuromasts from an untreated fish (Figure 4a) and one treated with 0.100g/L streptomycin (Figure 4b), which is roughly 10 times the amount used in previous studies (Montgomery et al. 2003). Both neuromasts have full rows of hair cells running down the middle of them. Consequently, the dose for the swim tests was not selected on the basis of this project's SEM study. Rather, the swim tests used a dose of 0.02 g/L streptomycin, well over the amount which Montgomery et al. (2003) had previously shown to disturb a trout's lateral line system.

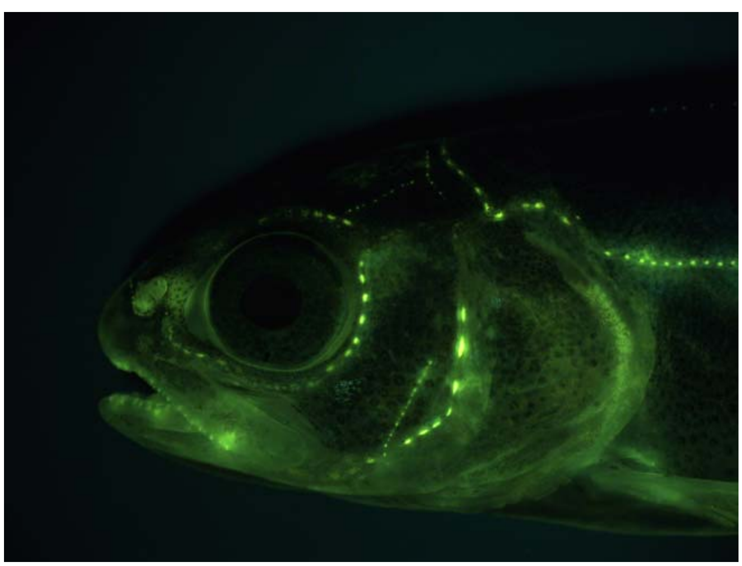

Figure 3. Steelhead treated with DASPEI and viewed with a fluorescent microscope. The lateral line is visible, composed of neuromasts (green dots).

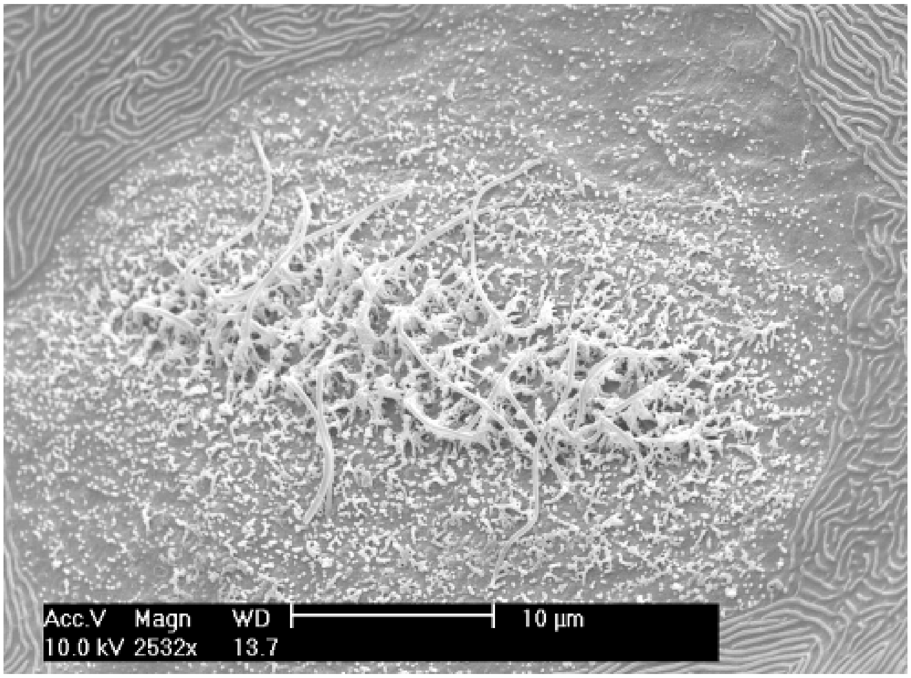

Figure 4a. Untreated superficial neuromast (no streptomycin)

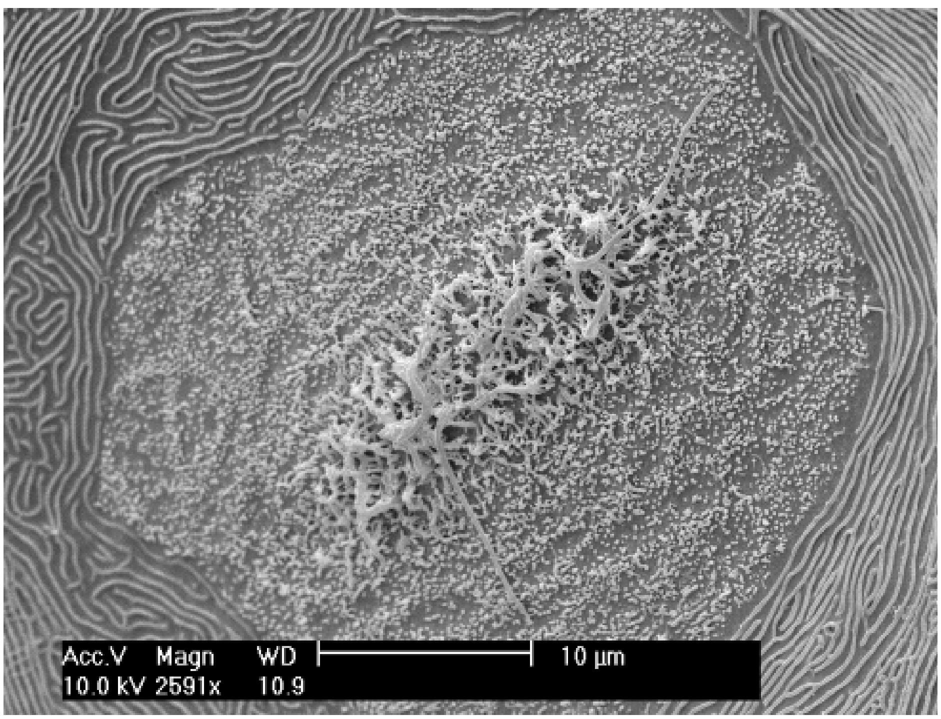

Figure 4b. Superficial neuromast treated with 0.100 g/L streptomycin

3.2. Results of the Swimming Trials

Fish contacted the screen during all treatments. A three-way ANOVA (analysis of variance) analyzing the effects of light level, screen vibrations, and streptomycin exposure found no significant differences between any of the treatments ($P > 0.05$). There was a large amount of individual variation present in the swimming performance trials of all treatments, most noticeably in the control group. Some steelhead chose not to swim and contacted the screen frequently or impinged on it, while others swam at the front of the chamber and never approached the screen. All the fish probably had the ability to continuously swim at 60 cm/s in the chamber, but some chose not to. The individual variation was greater than the variation caused by the treatments, adding to the lack of significance in the findings.

Some general trends, however, are still apparent in the results (Figure 5). Some fish appeared hesitant to swim, until after their initial contact(s) with the screen. Many of the contacts (57% during daytime runs and 67% during nighttime runs) occurred within the first minute of the trial. The mean contact rate during the night (7.7) was almost double that of the mean daytime contact rate (3.9). Under most conditions, the streptomycin-treated fish appear to have contacted the screen more frequently, with the exception of vibrating screens during the night.

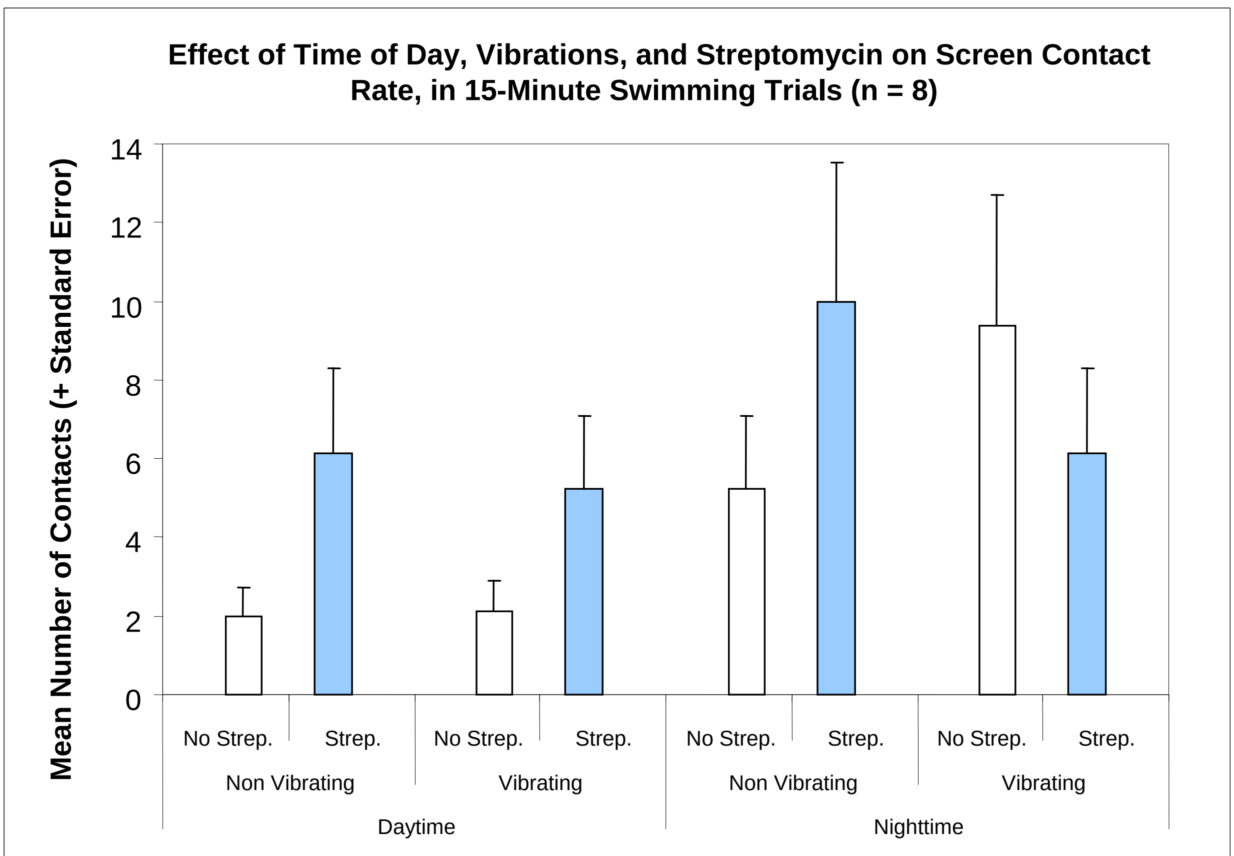

Figure 5. Effect of time of day, vibration, and streptomycin on screen contact rate, in 15-minute swimming trials (n = 8 per test group; total of 64 fish tested)

During the night the streptomycin-treated fish seemed to hit the screens with a greater fraction of their body during their initial contacts. Also, they commonly would burst-swim forward, away from the screen, much faster and farther than the non-treated fish. After a few of these contacts some fish would swim to the front and not re-touch the screen again.

Individual fish varied greatly in their swimming performance, and individual differences seemed more pronounced than those of the treatments in most treatment groups. For example, of the 8 fish tested during the day with no added vibrations or streptomycin, 4 of the fish never contacted the screens, but 2 fish became completely impinged and were no longer able to swim.

4.0 Conclusions and Recommendations

4.1. Conclusions

It is difficult to determine if streptomycin sulfate affects a steelhead's lateral line system. The SEM images show no indication that the fish's hair cells were damaged, even at 10 times the dose that has been shown to disrupt lateral line function in past studies (Montgomery et al. 2000). It is possible that the nitrogenous waste build in the buckets which occurred during the 24-hour treatments may have inactivated the streptomycin, causing it to have no effect on the steelhead's lateral line system. This problem could be lessened in future studies by food-depriving the fish for a couple days prior to experimentation so they excrete less waste during the streptomycin treatments. Another possibility is that the hair cells were damaged during the initial contact with the streptomycin and then regenerated during the 24-hour exposure period. Hair cells in larval fish can regenerate in 12 hours after exposure to antibiotics (Harris et al. 2003). Newly grown hair cells in adult fish are less susceptible to antibiotics and can even be grown in antibiotic-treated water (Song et al. 1995). Full recovery of hair cells following antibiotic exposure in adult fish, however, can take over 10 days (Coombs et al 2001). The time required for fish to regenerate a hair cell probably varies with species.

The swimming trials do show a trend for increased screen contacts for the streptomycin-treated fish, but the increase was not statistically significant. Because the streptomycin-treated fish appeared to contact the screen more severely and to swim in a more startled manner during the night, compared with the non-treated fish, it is possible that the streptomycin blocked some lateral line input to the fish, but by some other means than hair cell cleavage. An electro-physiological study, monitoring the neural responses in the lateral line system of fish treated with streptomycin compared to controls, would determine if the chemical is having an effect.

Juvenile steelhead trout appear to use both vision and mechanoreception during fish screen avoidance. Trends of more contacts occurred during the night than day and after streptomycin treatments compared to controls. Vision appears to be more important than mechanoreception in this species, because the contact rate was generally lower during the day.

The addition of vibrations at 45 hertz appeared to have very little effect on the fish's swimming performance. Other frequencies may invoke a greater response from the fish, and using non-constant, randomized bursts of vibrations may also be more effective. Other species may also respond to the vibrating stimulus to a greater extent. This study determined that trout have very few neuromasts in their lateral line system compared to cyprinids (minnows and carps) or centrarchids (sunfish and bass), and may not be as attuned to vibrational cues in their environment.

It is also possible that steelhead born and reared in natural settings would respond differently than captivity-reared fish to unusual vibrations in the water or the presence of a screen. The hatchery steelhead used in this experiment lived in large nonthreatening tanks, void of predators or challenging water velocities, for their entire lives. The lack of a challenging environment during development may explain why some individuals might have chosen not swim or to repeatedly contacted the fish screens during the experiment.

Other stimuli, such as strobe lights reflecting off the screen, may prove to be a more effective deterrent for a predominantly visual fish such as steelhead. Due to numerous delays in the construction and installation of the experimental flume, the strobe light trials could not be completed during the experimental period.

This important research will continue, using the equipment funded by this experiment, to address these and other questions over the next two years, to identify a strong behavioral deterrent to fish screens obscured by darkened conditions.

4.2. Recommendations

A large number of behavioral fish screen deterrents remain to be tested, both for steelhead and other native fishes. The vibrations emitted from the screen can be tested at different frequencies and amplitudes. Also, using non-constant, randomized bursts of vibrations may provide the perception of a greater threat or may effectively prolong fish habituation to the stimulus. The effect of strobe lights reflecting off a screen also may provide a strong visual deterrent to fish, particularly at night.

Some of the larger water-extraction facilities in California use louver arrays instead of fish screens to prevent fish from passing into intake structures. The effects of vibrating louver arrays may be more effective than vibrating screens. Experiments conducted in a controlled laboratory setting using small-scale louvers could evaluate this possibility.

Different species of fish can have vastly different abilities to perceive vibrations at different wavelengths and amplitudes. Other fish species of interest, such as Sacramento splittail or delta smelt, may show very different responses to added vibrations or lighting on screens. Therefore, tests should be conducted on many species of freshwater fish.

There is also concern for nearshore marine fishes, which can become entrained in the influent of coastal power plants that use once-through cooling technology. Similar studies to those conducted in this experiment can be used in a marine laboratory setting, to see if the common marine species show behavioral responses to vibrations or additional lighting.

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4.3. Benefits to California

California’s demand for water and electricity is expected to increase in the near future, increasing the need for water diversions and the concomitant threat of entrainment-related losses for many aquatic animals. Fish screens with improved deterrent capabilities will help minimize the impact of water diversions on California’s aquatic populations.

The equipment and ideas derived from this project will guide further research on an effective, close-proximity fish-screen deterrent. The specific knowledge gained about steelhead trout is particularly valuable, as steelhead represent an important, threatened gamefish, which can encounter many fish screens at industrial and agricultural water diversions during their migration to the ocean.

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